

## CLAIMS

What is claimed is:

- 5 1. An isolated nucleic acid molecule having a nucleic acid sequence encoding a transposon, wherein the nucleic acid sequence has a methylation at at least one nucleotide.
- 10 2. An isolated nucleic acid molecule according to Claim 1, further comprising a nucleic acid molecule encoding a desired gene.
- 15 3. An isolated nucleic acid molecule according to Claim 1, wherein said methylation is present at least C in a CG sequence.
- 20 4. An isolated nucleic acid molecule according to Claim 1, wherein said transposon is of a DNA-type.
- 25 5. An isolated nucleic acid molecule according to Claim 1, wherein said transposon belongs to Tc1/mariner types.
6. An isolated nucleic acid molecule according to Claim 1, wherein said transposon comprises *Sleeping Beauty*.
- 25 7. An isolated nucleic acid molecule according to Claim 2, wherein said desired gene is operably linked to said transposon, or is capable of being operably linked to said transposon when intracellularly introduced.
- 30 8. An isolated nucleic acid molecule according to Claim 1 for use in introducing a foreign gene into a host.

9. An isolated nucleic acid molecule according to Claim 8, wherein said host comprises a eukaryote.
10. An isolated nucleic acid molecule according to Claim 8, wherein said host comprises a mammal.
11. An isolated nucleic acid molecule according to Claim 8, wherein said host comprises a rodent.
- 10 12. An isolated nucleic acid molecule according to Claim 1, wherein a transposase functions at a location on a genome to which said nucleic acid molecule is inserted.
- 15 13. A gene cassette having a nucleic acid sequence encoding a transposon, wherein said nucleic acid sequence has a methylation at at least one nucleotide.
- 20 14. A vector having a nucleic acid sequence encoding a transposon and a nucleic acid sequence encoding a desired gene, wherein said nucleic acid sequence has a methylation at at least one nucleotide.
15. A vector according to Claim 14, wherein said methylation is present at least C in a CG sequence.
- 25 16. A vector according to Claim 14, wherein said transposon is of a DNA-type.
- 30 17. A vector according to Claim 14, wherein said transposon belongs to *Tc1/mariner* types.
18. A vector according to Claim 14, wherein said transposon comprises *Sleeping Beauty*.

19. A vector according to Claim 14, wherein said desired gene is operably linked to said transposon, or is capable of being operably linked to said transposon when 5 intracellularly introduced.
20. A vector according to Claim 14 for use in introducing a foreign gene into a host.
- 10 21. A vector according to Claim 20, wherein said host comprises a eukaryote.
22. A vector according to Claim 20, wherein said host comprises a mammal.
- 15 23. A vector according to Claim 20, wherein said host comprises a rodent.
24. A vector according to Claim 14, wherein a 20 transposase functions at a location on a genome to which said nucleic acid molecule is inserted.
25. A composition for rendering a transposase to act on a foreign nucleic acid molecule to be inserted on a genome, 25 wherein said composition comprises a nucleic acid sequence encoding a transposon and a nucleic acid sequence encoding a desired gene, wherein said nucleic acid sequence has a methylation at at least one nucleotide.
- 30 26. A cell comprising a nucleic acid sequence encoding a transposon and a nucleic acid sequence encoding a desired gene, wherein said nucleic acid sequence has a methylation at at least one nucleotide.

27. A cell according to Claim 26, wherein said methylation is present at least C in a CG sequence.

5 28. A cell according to Claim 26, wherein said transposon is of a DNA-type.

29. A cell according to Claim 26, wherein said transposon belongs to Tc1/mariner types.

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30. A cell according to Claim 26, wherein said transposon comprises *Sleeping Beauty*.

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31. A cell according to Claim 26, wherein said desired gene is operably linked to said transposon.

32. A cell according to Claim 26 for use in introducing a foreign gene into a host.

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33. A cell according to Claim 26, wherein said host comprises a eukaryote.

34. A cell according to Claim 26, wherein said host comprises a mammal.

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35. A cell according to Claim 26, wherein said host comprises a rodent.

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36. A tissue comprising a nucleic acid sequence encoding a transposon and a nucleic acid sequence encoding a desired gene, wherein said nucleic acid sequence has a methylation at at least one nucleotide.

37. A tissue according to Claim 36, wherein said methylation is present at least C in a CG sequence.

38. A tissue according to Claim 36, wherein said 5 transposon is of a DNA-type.

39. A tissue according to Claim 36, wherein said transposon belongs to *Tcl*/mariner types.

10 40. A tissue according to Claim 36, wherein said transposon comprises *Sleeping Beauty*.

41. A tissue according to Claim 36, wherein said desired gene is operably linked to said transposon.

15 42. A tissue according to Claim 36 for use in introducing a foreign gene into a host.

43. A tissue according to Claim 42, wherein said tissue 20 comprises a eukaryotic tissue.

44. A tissue according to Claim 42, wherein said tissue comprises a mammalian tissue.

25 45. A tissue according to Claim 42, wherein said tissue comprises a rodent tissue.

46. A biological organism comprising a nucleic acid sequence encoding a transposon and a nucleic acid sequence 30 encoding a desired gene, wherein said nucleic acid sequence has a methylation at at least one nucleotide.

47. A biological organism according to Claim 46, wherein

said methylation is present at least C in a CG sequence.

48. A biological organism according to Claim 46, wherein said transposon is of a DNA-type.

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49. A biological organism according to Claim 46, wherein said transposon belongs to Tc1/mariner types.

50. A biological organism according to Claim 46,  
10 wherein said transposon comprises *Sleeping Beauty*.

51. A biological organism according to Claim 46, wherein said desired gene is operably linked to said transposon.

15 52. A biological organism according to Claim 46, which comprises a eukaryote.

53. A biological organism according to Claim 46, which comprises a mammal.

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54. A biological organism according to Claim 46, which comprises a rodent.

55. A biological organism according to Claim 46, wherein  
25 said desired gene is not derived from said biological organism.

56. A method for producing a transgenic biological organism, comprising the steps of:

30 A) providing an isolated nucleic acid molecule having a nucleic acid sequence encoding a transposon;  
B) transforming a germ cell of a desired biological organism with said nucleic acid molecule;

C) selecting an individual in which the germ cell has a methylation in a nucleic acid sequence encoding said transposon; and

5 D) regenerating a biological organism using the transformed germ cell.

57. A method according to Claim 56, wherein said biological organism comprises a eukaryote.

10 58. A method according to Claim 56, wherein said biological organism comprises a mammal.

59. A method for producing a transgenic biological organism comprising the steps of:

15 A) providing an isolated nucleic acid molecule having a nucleic acid sequence encoding a transposon, wherein the nucleic acid sequence has a methylation at at least one nucleotide;

20 B) transforming a germ cell of a desired biological organism with said nucleic acid molecule; and

C) regenerating a biological organism using the transformed germ cell.

60. A method according to Claim 59, wherein said 25 biological organism comprises a eukaryote.

61. A method according to Claim 59, wherein said biological organism comprises a mammal.

30 62. A method according to Claim 59, wherein said biological organism comprises a rodent.

63. A kit for producing a transgenic biological organism,

comprising:

5       A) an isolated nucleic acid molecule having a nucleic acid sequence encoding a transposon, wherein the nucleic acid sequence has a methylation at at least one nucleotide; and

      B) a transposase.

10      64. A kit according to Claim 63, further comprising instructions indicating a method for use of said nucleic acid molecule and transposase.

15      65. Use of an isolated nucleic acid molecule having a nucleic acid sequence encoding a transposon, wherein the nucleic acid sequence has a methylation at at least one nucleotide for producing a transgenic biological organism.

20      66. A nucleic acid fragment comprising a nucleic acid sequence located between at least two inverted repeat sequences, wherein the inverted repeat sequences have the capability of binding to a transposase, wherein the nucleic acid fragment is capable of being incorporated into a DNA in a cell, wherein at least one nucleotide thereof is methylated.

25      67. A nucleic acid fragment according to Claim 66, wherein the nucleic acid sequence comprises at least a portion of a foreign gene.

30      68. A nucleic acid fragment according to Claim 66, wherein said nucleic acid sequence comprises at least one expression controlling region.

69. A nucleic acid fragment according to Claim 68, wherein the expression controlling region is selected from the group consisting of a promoter, enhancer and silencer.

5 70. A nucleic acid fragment according to Claim 66, further comprising at least a portion of a foreign gene, wherein the nucleic acid sequence is operably linked to a sequence encoding at least a portion of the foreign gene.

10 71. A nucleic acid fragment according to Claim 66 wherein said cell is derived from an animal.

72. A nucleic acid fragment according to Claim 71, wherein said cell is obtained from a vertebrate.

15 73. A nucleic acid fragment according to Claim 72, wherein the vertebrate is a mammal.

74. A nucleic acid fragment according to Claim 73, 20 wherein the mammal is a primate or a rodent.

75. A nucleic acid fragment according to Claim 66, the DNA of the cell is selected from the group consisting of a cellular genome, episome and plasmid.

25 76. A nucleic acid fragment according to Claim 66, wherein said at least one inverted repeat sequence comprising the sequence set forth in SEQ ID NO: 20 or 21, or a portion thereof.

30 77. A nucleic acid fragment according to Claim 66, wherein said transposase is SB protein.

78. A nucleic acid fragment according to Claim 77, wherein the transposase has at least 80 % amino acid homology to the sequence set forth in SEQ ID NO: 3.

5 79. A nucleic acid fragment according to Claim 66, wherein said at least one inverted repeat sequence comprises at least one tandem repeat sequence, and the tandem repeat sequence comprises a nucleotide sequence set forth in SEQ ID NO: 26 or that having at least 80%  
10 homology to the sequence set forth in SEQ ID NO: 26.

80. A nucleic acid fragment according to Claim 66, wherein said at least one inverted repeat sequence comprises at least one tandem repeat sequence, wherein  
15 the tandem repeat sequence is selected from the group consisting of nucleic acid sequences set forth in SEQ ID NOs: 22-25.

81. A nucleic acid introduction system for introducing  
20 into a DNA of a cell another DNA, the system comprising:

25 A) a nucleic acid fragment comprising a nucleic acid sequence located between at least two inverted repeat sequences, wherein the inverted repeat sequences have the capability of binding to a transposase, wherein the nucleic acid fragment is capable of being incorporated into a DNA in a cell, wherein at least one nucleotide thereof is methylated; and

30 B) a transposase or a nucleic acid encoding a transposase.

82. A nucleic acid introduction system according to Claim 81, therein the transposase is SB protein.

83. A nucleic acid introduction system according to  
Claim 81, wherein said transposase has the amino acid  
sequence set forth in SEQ ID NO:3 or a variant thereof,  
or the nucleic acid sequence encoding the transposase has  
5 the nucleic acid sequence set forth in SEQ ID NO: 2 or  
a variant thereof.

84. A nucleic acid introduction system according to  
Claim 81, wherein said nucleic acid encoding the  
10 transposase is incorporated into the cellular genome.

85. A nucleic acid introduction system according to  
Claim 81, further comprising a plasmid or a virus vector,  
wherein said plasmid or virus vector comprises the nucleic  
15 acid fragment as a part thereof.

86. A nucleic acid introduction system according to  
Claim 81, wherein nucleic acid fragment comprises at least  
a portion of a sequence encoding a foreign gene.  
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87. A nucleic acid introduction system according to  
Claim 81, wherein the nucleic acid fragment is introduced  
into the cell by means of a method selected from the group  
consisting of particle bombardment; electroporation;  
25 microinjection; use of a gene introduction reagent; and  
use of a virus vector.

88. A method for producing a transgenic biological  
organism, comprising the steps of:  
30 introducing a nucleic acid fragment and transposase  
into a pluripotent cell, wherein the nucleic acid fragment  
comprises a nucleic acid sequence located between at least  
two inverted repeat sequences, wherein the inverted repeat

sequences have the capability of binding to a transposase, wherein the nucleic acid fragment is capable of being incorporated into a DNA in a cell, wherein at least one nucleotide thereof is methylated; and

5 growing the cell into a living body.

89. A method according to Claim 88, wherein said pluripotent cell is selected from the group consisting of an oocyte, an embryo, an egg and a stem cell.

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90. A method according to Claim 88, wherein said biological organism is a rodent or a primate.

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91. A method according to Claim 89, wherein said biological organism is a mouse or a rat.

92. A method for introducing a nucleic acid into a DNA of a cell, comprising the step of:

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introducing a nucleic acid fragment into a cell, wherein the nucleic acid fragment comprises a nucleic acid sequence located between at least two inverted repeat sequences, wherein the inverted repeat sequences have the capability of binding to a transposase, wherein the nucleic acid fragment is capable of being incorporated into a DNA in a cell, wherein at least one nucleotide thereof is methylated.

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93. A method according to Claim 92 further comprising the step of introducing a transposase into the cell.

94. A method according to Claim 92, wherein the transposase has at least 80 % homology to the sequence set forth in SEQ ID NO: 3.

95. A method according to Claim 92, wherein said cell comprises a nucleic acid encoding the transposase.

5 96. A method according to Claim 95, wherein the nucleic acid encoding the transposase is incorporated into the cellular genome.

10 97. A method according to Claim 95, wherein the transposase is stably expressed in the cell.

98. A method according to Claim 95, wherein the transposase is operably linked such that it is under the control of an inducible promoter.

15 99. A method according to Claim 92, wherein the nucleic acid sequence encodes a protein.

20 100. A method according to Claim 92, wherein the nucleic acid sequence encodes a marker protein.

101. A method for tranposing a nucleic acid sequence in a cell, comprising the steps of:

25 introducing a transposase into a cell comprising a nucleic acid fragment, wherein the nucleic acid fragment comprises a nucleic acid sequence located between at least two inverted repeat sequences, wherein the inverted repeat sequences have the capability of binding to a transposase, wherein the nucleic acid fragment is capable of being 30 incorporated into a DNA in a cell, wherein at least one nucleotide thereof is methylated;

wherein the transposase transposes the nucleic acid sequence from a first location in a DNA of the cell to

a second location of the DNA.

102. A method according to Claim 101, wherein the DNA of the cell is a genomic DNA.

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103. A method according to Claim 101, wherein the first location is an extrachromosomal DNA.

104. A method according to Claim 101, wherein the second location is an extrachromosomal DNA.

105. A method according to Claim 101, wherein the transposase introduces a nucleic acid into the cell.

15 106. A method for identifying a gene in a cell, comprising the steps of:

introducing into a cell, a nucleic acid fragment comprises a nucleic acid sequence located between at least two inverted repeat sequences, wherein the inverted repeat 20 sequences have the capability of binding to a transposase, wherein the nucleic acid fragment is capable of being incorporated into a DNA in a cell, wherein at least one nucleotide thereof is methylated, and a transposase;

25 digesting the DNA in the cell with a restriction endonuclease capable of digesting the nucleic acid sequence;

identifying the inverted repeat sequence;  
determining a sequence of a nucleic acid having a similar sequence to the inverted repeat sequence; and  
30 comparing the sequence with sequence information in a sequence information database.